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ORIGINAL PAPER

Influence of leaf removal on grape, wine and aroma compounds of *Vitis vinifera* L. cv. Merlot under Mediterranean climate

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Abstract

Merlot is one of the most cultivated cultivars in the world since it easily adapts to different climatic conditions. Leaf removal (LR) is commonly used for red berry varieties but in cold wine-growing areas and nothing has been reported on the effects of LR on Merlot cultivated under Mediterranean climate. The aim of the research is to evaluate the influence of this technique on grape and wine quality as well as wine aroma potential of Merlot cultivated in Sicily. Vines were subjected to LR for two consecutive years, and productivity and chemical parameters were monitored in grapes, whereas chemical composition and volatile aroma compounds, analyzed by gas chromatography mass spectrometry, were monitored in wines. LR positively influenced the plant yield, increased the sugar content and decreased the acidity in grapes at harvest. The wines of the defoliated treatment showed a higher content of total polyphenols and anthocyanins, higher color intensity, and lower color hue. Merlot wines obtained under Mediterranean climate were characterized by a high amount of esters and varietal aromas and the content of most of volatiles were even increased by the LR with positive effects on the aroma potential of Merlot wines. The vintage affected almost all the studied parameters with the warmer and dryer vintage enhancing the LR effects on grapes and wines. This is of great interest in the light of the climate changes towards the global warming and the increasing aridity of the Mediterranean area.

Keywords Aroma compounds · Leaf removal · Mediterranean climate · Merlot cultivar · Wine composition

Introduction

Vineyards are subjected to many management practices including row orientation and spacing, soil surface and manipulation of the canopy structure such as density, clipping, pruning, and tilling. The leaf removal (LR), or defoliation, carried out in different periods of the vegetative cycle of the plant, usually after flowering, between setting and veraison, is an agronomic practice that is becoming increasingly common in viticulture. It is often used in cold wine-growing areas and for red berry varieties. This technique consists in the elimination of the leaves that cover the bunches improving aeration and sun exposure; several factors such as the timing of the agronomic treatment, the apical or basal position of the LR, the manual or mechanical application of the technique and finally the harvest time of the grapes, must be considered for the treatment effectiveness.

The removal of the basal leaves, close to the cluster, improves some aspect linked to the health of the grapes, such as a lower risk of fungal attacks due to a higher ventilation and a greater effectiveness of phytosanitary treatments. At the same time, the higher sun exposure leads to a better technological maturation of the grapes, a greater accumulation of sugars and a lower titratable acidity, promotes a better phenolic complexity but above all increase flavonoid and anthocyanin concentration [1, 2]; advantages on the

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volatile compounds responsible for the wine aroma of different cultivars such as Nero d'Avola, Tempranillo, Shyraz, Semillon, Cabernet franc, and Petit Verdot [3–7] have been demonstrated, too.

The red wine grape (*Vitis vinifera* L.) cultivar Merlot is an early ripening variety of dark blue wine grapes, which is used both as mono-varietal and blending grape wine. Its softness and fleshiness make Merlot grape to be blended with Cabernet Sauvignon later ripening and rich in tannins [8]. The exact origin of Merlot is already unknown, but genetic similarity to Cabernet Franc and Carmenere has been demonstrated [9]. Merlot is one of the most popular red wine varieties on the market; its aroma is reminiscent of red berry fruits ranging from fresh to jammy. Merlot is among the most cultivated cultivars in the world with a vineyard area of about 260,000 hectares. In Italy about 25,000 hectares are dedicated to the cultivation of Merlot [10]. Recently, different winemaking practices have been tried to improve Merlot wine quality in relation to wine aroma such as the use of partially dehydrated grapes, low fermentation temperature, and different yeasts [11–13]. As regards the impact of LR on Merlot cultivar, researches had been carried out on grapes and wines mainly under cold climates, limited to the anthocyanin, polyphenol, and tannin concentration [14–18]. At the best of our knowledge, no information is present in literature about the effects of LR on Merlot cultivated under Mediterranean climate, characterized by climate with rainy winters and dry and hot summers; moreover, the influence of this agronomic practice on the volatile aroma compounds has never been investigated. Because the wine aroma profile is one of the main aspects related to the quality of the final product, it should be taken into high consideration in the effectiveness assessment of an agronomic technique. Thus, this research aimed to evaluate how the practice of LR, carried out in Mediterranean areas, can affects the quality of Merlot grapes and wine with particular attention to the aroma compounds.

Materials and methods

Samples

The research was carried out in two subsequent production years (2016 and 2017) on Merlot vines grafted onto 140 Ru (*V. berlandieri* × *V. rupestris*) in an experimental rainfed vineyard of about 1.5 ha, located in the territory of Monreale, C/da Roano-Grisì (Palermo, Sicily, Italy) (37° 56′ 39.2″ N, 13°05′ 33.56″ E). The vines were planted in 2008 at a density of 4000 plants/ha. The vineyard was cultivated as espalier with spurred cordon pruning (five spurs per plant, carrying two buds each), and north–south oriented. A vineyard portion of 1000 m^2 was used for the experiment. A fully randomized experimental design consisted of ten blocks, five for control (non-defoliated) and five for LR, was used (Fig. 1S). The LR was made manually immediately after the setting, when the berries were groat-sized (BBCH-scale: stage 7, code 73), removing the first 4–5 basal leaves of each branch on both sides of the row and gaming so a good exposure of each single bunch. As regard the control, the vine management was the same as that of defoliated vines (namely same number of spurs per plant, same number of buds per spur), except for leaf removal.

Starting 15 days after veraison, 250 berries per each treatment were randomly collected, every 5 days, from different positions of the blocks and used for the technical (sugar, titratable acidity, and pH) and phenolic (anthocyanins and flavonoids) maturity assessment [19]. Yield at harvest was determined on ten marked vines per experimental block. Once ripened, the grapes of each treatment were harvested and immediately transported to the winery of the Regional Institute of Wine and Oil (IRVOS) in Marsala (Sicily, Italy), where they were pressed. The musts from the two treatments (control and LR) were subjected to three separated vinifications: they were added of dry yeast NDA21 (0.3 g/L), fermented for 9 days at 26–28 °C in contact with the skins, then they were sulfated at 0.05 g/L. Malo-lactic fermentation followed, by the addition of biomass (0.005 kg/L dregs). After setting, the wines were placed in dark bottles and stored in a conditioned room at 18–20 °C and 70–80% relative humidity until analyses.

A total of 72 wine bottles, 18 bottles for each treatment and each vintage, were selected for the analyses, carried out in duplicate after 6 months from bottling.

Climatic conditions

The territory of Monreale (Palermo, Sicily, Italy) is characterized by a Mediterranean climate (Köppen climate classification Csa) with hot, dry summers and mild, wet winters. In the years 2016 and 2017, rainfall registered from vegetative period (April–August) was 163 mm and 122 mm for 2016 and 2017, respectively, whereas the average temperatures were 19.2 °C ($T_{\min}$) and 27.5 °C ($T_{\max}$) for 2016, and 19.1 °C ($T_{\min}$) and 29.2 °C ($T_{\max}$) for 2017 (Fig. 2S).

Chemical analysis

The physicochemical parameters in grapes and wines were determined according to the EEC Official Method [19].

Volatile extraction and analysis

The volatile aroma profile of the Merlot wines was investigated following the method previously developed which includes Headspace Solid-Phase Microextraction technique (HS-SPME) followed by Gas Chromatography analysis coupled online with Mass Spectrometry (GC–MS) [3, 20]. Exactly, a 40 mL vial was filled with 20 mL of wine sample; the extraction was performed in the headspace vial using a DVB/CAR/PDMS (Divinylbenzene/Carboxen/Polydimethylsiloxane) fiber of 50/30-µm film thickness housed in its manual holder (Supelco, Bellefonte, PA, USA). The vial was kept at 30 °C, the sample was continuously stirred, equilibrated for 15 min and extracted for 20 min. After sampling, the SPME fiber was introduced onto the splitless injector of the gas chromatographer, kept at 260 °C, for 3 min for thermal desorption of the analytes. The volatile analysis was performed with a Shimadzu GC 2010 Plus gas chromatographer directly interfaced with a TQMS 8040 triple quadrupole mass spectrometer (Shimadzu, Milan, Italy). The conditions were as follows: capillary column, VF-WAXms, 60 m, 0.25 mm i.d., 0.25 µm film thickness polar column (Agilent Technologies Italia S.p.A., Milan, Italy); oven temperature, 45 °C held for 5 min, increased to 80 °C at 10 °C/min and to 240 °C at 2 °C/min; carrier gas, helium at a constant flow of 1 mL/min; transfer line temperature, 250 °C; acquisition range, 40–200 *m/z*; scan speed, 1250 amu/s.

Each compound was identified using mass spectral data, NIST'18 (NIST/ EPA/NIH Mass Spectra Library, version 2.0, USA), FFNSC 3.0 database, linear retention indices, literature data and the injection of standards, where available, as reported by Cincotta et al. [21]. The volatile compounds thus extracted and identified have been quantified using the Standard Addition method, as previously reported [3]; standards were purchased from Sigma–Aldrich s.r.l. (Milan, Italy) at the highest purity available.

Odour activity value

The Odour Activity Value (OAV) was calculated by dividing the concentration of a specific volatile compound by its odour perception threshold found in the literature [3, 22–25] to evaluate the contribution of each chemical compound to the aroma of Merlot wine.

Statistical analysis

The XLStat software, version 2019.1.2 (Addinsoft, Damremont, Paris, France) was used to evaluate statistically the results. Two-way ANOVA (treatment and year), and Principal Component Analysis (PCA) were performed on the data to investigate the differences among samples from different treatments (Control and LR) and different vintages. The model was statistically significant with a p value < 0.05 or less.

Results

Table 1 shows the productivity data of the vines and the physicochemical parameters of the grapes at harvest. Statistical elaboration of the data showed that the LR slightly increased ($p < 0.05$) yield in both vintages via a higher weight of bunches and berries, whereas no statistically significant differences were observed in the number of bunches

Table 1 Productivity data and physicochemical parameters of the Merlot grapes at harvest

	2016		2017		ANOVA significance	
	Control	LR[a]	Control	LR	Tr[b]	Y[c]
Yield (kg/plant)	2.766	3.233	2.514	3.007	*	**
Number of bunches per plant	22.7	21.1	20.3	18.9	ns[d]	*
Bunch weight (g)	125.8	148.6	123.8	151.4	*	ns
Berry weight (g)	0.91	1.11	0.87	1.19	*	ns
°Babo	20.04	21.28	19.82	21.08	*	ns
pH	3.51	3.57	3.53	3.59	ns	ns
Titratable acidity (g/L)	5.9	5.3	5.1	4.6	*	*

[a] Leaf removal

[b] Treatment

[c] Year

[d] Non-significant

*Data that exhibited statistically significant differences at $p < 0.05$

**Data that exhibited statistically significant differences at $p < 0.01$

per plant due to the treatment. Also, the vintage had a significant influence on yield, with the lowest number of bunches per plant in the 2017 vintage. As regards the physicochemical parameters of the grapes, the sugar content was affected only by treatment, resulting higher ($p < 0.05$) in the defoliated samples, whereas the titratable acidity showed statistically differences both between years and treatments, with the lowest values in the defoliated samples from 2017 vintage; finally, no statistically significant differences were observed in pH values neither between treatments nor vintages.

Table 2 reports the physicochemical parameters of the Merlot wines; the statistical analysis revealed that they were affected both by the treatment and the vintage. LR allowed to obtain wines with lower total acidity and higher alcohol, total anthocyanin and total polyphenol content, higher values for color intensity and lower for color hue. The effects of LR on the physicochemical parameters of wine were enhanced in 2017 vintage.

Table 3 reports the identified volatile compounds in Merlot wine samples along with the calculated LRI, the quantitative data, the odour thresholds, and the odour descriptors. Globally 54 volatile aroma compounds were identified, most of which for the first time in Merlot wines.

Esters were the chemical class with the highest number of identified compounds, followed by those of alcohols, acids, aldehydes, and terpenes. Among esters, ethyl octanoate, ethyl decanoate, ethyl hexanoate, and isoamyl alcohol were the most represented. All showed a double amount in the samples from defoliated treatment; moreover, among esters, the amount of ethyl esters was positively affected in 2017 vintage.

Alcohols were the class of compound quantitatively most represented; isoamyl, benzyl and β-phenylethyl alcohols showed statistical differences between the two treatments, with the highest ($p < 0.05$) amount in the defoliated samples. Aliphatic aldehydes, namely octanal, nonanal, and decanal, were present in our samples but with an amount inferior to the quantification limit in both treatments. Linear and branched fatty acids from C_4 to C_8 were also identified, in a higher amount in defoliated samples. The content of aliphatic aldehydes, fatty acids, and alcohols did not statistically differ between vintages, except for hexanol whose amount was higher in 2017.

Following esters, terpenes were the class of compounds qualitatively most represented; most of them, especially the oxygenated ones, were affected by both treatment and vintage. The highest amounts ($p < 0.05$ or $p < 0.01$) of terpenes were observed in wine samples produced in the 2017 from defoliated vines.

Figure 1 reports the HS-SPME–GC–MS chromatogram in SIM mode ($m/z = 93 + 121.0 + 136.0$) of the identified terpenes, key aroma compounds in Merlot wines. The main terpenes were p-cymene, linalool, β-terpineol, α-citronellol, and (*E*)-nerolidol.

To understand which compounds contributed to the aromatic bouquet of the wine, the OAVs were calculated and those ≥ 0.5 were reported in Table 4. In both treatments, only twelve compounds had an OAV ≥ 0.5 in the two vintages, namely eight esters, three alcohols, and one terpene. Among esters, ethyl hexanoate (fruity, green apple) had the highest OAV value, followed by ethyl 3-methylbutanoate (fruity, apple) mainly in the samples from the defoliated treatment of the 2017 vintages.

The influence of LR and vintage on that volatile aroma compounds was further studied by applying the principal components analysis (PCA) to volatile compounds with OAV ≥ 0.5. The first two principal components accounted for more than 99% of total variance (89.16% for PC1 and

Table 2 Physicochemical and chemical parameters (average value of 18 samples, analysed in duplicate) of Merlot wines

	2016		2017		ANOVA significance	
	Control	LR[a]	Control	LR	Tr[b]	Y[c]
Alcohol (%vol)	13.2	14.8	13.4	15.2	*	ns
pH	3.59	3.68	3.53	3.62	ns[d]	ns
Total acidity (g/L)	5.50	4.99	4.90	4.41	*	*
Total anthocyanins (mg/L)	525	557	549	603	*	*
Total polyphenols (mg/L)	2451	3436	2739	3992	**	*
Color intensity (A420 + A520 + A620)	16.1	18.7	18.7	22.1	*	*
Color hue (A420/A520)	0.51	0.45	0.45	0.39	*	*

[a] Leaf removal

[b] Treatment

[c] Year

[d] Non-significant

*Data that exhibited statistically significant differences at $p < 0.05$

**Data that exhibited statistically significant differences at $p < 0.01$

Table 3 Volatile compounds identified and quantified (average value of eighteen samples, analyzed in duplicate) in the Merlot wine samples

Compounds	LRI[a]	2016		2017		ANOVA significance		Odour threshold[e] (μg/L)	Odour descriptor[f]
		Control	LR[b]	Control	LR	Tr[c]	Y[d]		
Esters (μg/L)									
Ethyl butanoate	1036	32.4	54.3	39.9	62.3	*	*	400	Fruity, strawberry
Ethyl 2-methylbutanoate	1051	22.5	34.2	31.1	43.3	*	*	18	Strawberry, candy fruit
Ethyl 3-methylbutanoate	1065	30.2	36.4	36.2	41.9	ns[g]	*	1	Fruity, apple
Isoamyl acetate	1115	371.0	644.9	368.9	642.8	*	ns	30	Banana
Methyl hexanoate	1180	10.9	15.7	10.8	15.4	ns	ns	–[h]	Pineapple, fruity, apple
Ethyl hexanoate	1226	894.7	1788.3	1315.4	2227.2	*	*	14	Fruity, green apple
Hexyl acetate	1266	27.0	48.9	26.1	49.7	*	ns	670	Fruity, herbs, apple, pear, cherry
Ethyl heptanoate	1326	15.7	58.5	19.4	63.2	*	*	220	Pineapple, fruity, apple
Methyl octanoate	1385	14.5	38.2	14.7	37.7	*	ns	200	Fruity, citric
Ethyl octanoate	1434	3841.8	7426.1	4737.9	9961.2	*	*	580	Fruity, candy, pineapple, pear, floral
Isoamyl hexanoate	1455	42.4	168.8	42.6	170.0	*	ns	1000	Sweet, fruity
(E)-4-Ethyl octanoate	1482	6.3	19.2	10.0	23.4	*	*	–	–
Ethyl nonanoate	1531	51.0	148.9	62.4	160.3	*	*	1300	Fruity, floral
Butyl octanoate	1547	7.8	15.1	7.6	15.6	*	ns	700	Fruity
Methyl decanoate	1591	7.9	22.0	7.8	22.2	*	ns	1.2	Wax, soapy, fruity
Ethyl decanoate	1636	1792.8	4256.2	2106.3	4987.4	*	*	200	Fruity, grape
Isoamyl octanoate	1654	51.9	68.9	51.8	69.7	*	ns	125	Wax, soapy, pear
Diethyl succinate	1672	558.9	1203.7	560.0	1209.4	*	ns	200,000	Wine, caramel, fruity
(E)-Ethyl-4-decenoate	1686	48.5	82.4	58.5	98.6	*	*	–	Fruity, green
β-Phenylethyl acetate	1812	28.2	55.1	28.3	54.6	*	ns	250	Roses, floral, honey
Ethyl dodecanoate	1837	137.2	177.4	153.2	241.4	*	*	1500	Candy, floral, waxy, soap
Isoamyl decanoate	1857	15.2	19.9	15.3	20.5	ns	ns	–	Fruity
Ethyl tetradecanoate	2045	6.3	12.9	12.8	21.6	*	*	2000	Waxy
All		*8014.8*	*16,396.1*	*9716.9*	*20,239.4*	*	*		
Alcohols (mg/L)									
Butanol	1150	0.03	0.04	0.03	0.05	ns	ns	150,000	Medical
Isoamyl alcohol	1213	2.06	3.11	2.10	3.15	*	ns	30,000	Burnt, alcohol, nail polish, whiskey
4-Methyl-1-pentanol	1298	0.07	0.09	0.07	0.11	ns	ns	50,000	Almond, toasted
3-Methyl-1-pentanol	1322	0.06	0.09	0.06	0.09	ns	ns	50,000	Vinous, herbaceous, cacao
Hexanol	1347	0.05	0.09	0.09	0.13	ns	*	110	Herbaceous, fatty, resinous
Octanol	1550	0.06	0.10	0.06	0.10	ns	ns	120	Intense citrus, roses
Benzyl alcohol	1862	0.83	1.14	0.85	1.16	*	ns	200,000	Candy, fruity
β-Phenylethyl alcohol	1904	8.21	11.47	8.09	11.45	*	ns	14,000	Roses, honey
All		*11.37*	*16.13*	*11.35*	*16.24*	*	ns		
Aldehydes (mg/L)									
Nonanal	1392	tri	tr	tri	tr	ns	ns	1	Green, slightly pungent
Decanal	1503	tr	tr	tr	tr	ns	ns	1	Grassy, orange skin-like
Dodecanal	1752	tr	tr	tr	tr	ns	ns	2	Floral, waxy
Acids (mg/L)									
2-Methyl propanoic acid	1565	0.08	0.13	0.10	0.15	ns	ns	200,000	Cheese
Butanoic acid	1648	0.14	0.21	0.16	0.21	*	ns	10,000	Cheese
3-Methyl butanoic acid	1794	0.03	0.11	0.03	0.09	*	ns	3000	Rancid, acidic
Octanoic acid	2053	0.13	0.17	0.11	0.19	*	ns	500	Rancidity, candy, cheese, animal, spice, unpleasant
Decanoic acid	2268	0.04	0.04	0.04	0.04	ns	ns	1000	Unpleasant, rancid fat, animal
All		*0.42*	*0.66*	*0.44*	*0.68*	*	ns		

Table 3 (continued)

Compounds	LRI[a]	2016		2017		ANOVA significance		Odour threshold[e] (μg/L)	Odour descriptor[f]
		Control	LR[b]	Control	LR	Tr[c]	Y[d]		
Terpenes (μg/L)									
β-Pinene	1105	tr	tr	tr	tr	ns	ns	140	–
3-Carene	1146	tr	tr	tr	tr	ns	ns	44	Mango leaf-like, sweet, green
α-Terpinene	1167	0.52	2.50	0.48	2.50	*	ns	–	–
Limonene	1188	0.57	0.91	0.63	1.09	ns	ns	15	Lemon, orange
(Z)-β-Ocimene	1236	0.56	0.14	0.64	0.26	*	*	1800	Candy, herbaceous
p-Cymene	1265	3.60	4.00	4.00	4.60	*	*	11.40	Fruity, sweet
Terpinolene	1277	0.28	0.39	0.32	0.41	ns	*	250	Oil, anise, mint
Citronellal	1473	0.18	0.29	0.22	0.31	ns	ns	–	Strong, citrus green
α-Copaene	1487	tr	tr	tr	tr	ns	ns	–	–
Linalool	1515	5.30	6.00	6.10	7.00	**	**	6	Fruity, citric
β-Terpineol	1540	10.10	15.00	11.90	17.20	**	**	110–400	–
α-Terpineol	1693	0.40	0.32	0.80	0.68	ns	**	250	Floral, candy, anise, mint
α-Citronellol	1756	6.30	7.80	7.70	9.20	**	**	100	Green, lemon
Geranyl acetone	1849	2.90	1.30	3.50	1.90	**	**	60	Floral
(E)-Nerolidol	2032	2.40	4.40	3.60	6.20	**	**	700	Rose, apple, green, citrus
All		*33.11*	*43.05*	*39.89*	*51.35*	**	**		

[a] Linear retention indices calculated on CP-Wax 52 CB column according to Van den Dool and Kratz equation

[b] Leaf removal

[c] Treatment

[d] Year

[e,f] Odour thresholds and odour descriptors are reported in the literature [3, 22–25]

[g] Non-significant

*Data that exhibited statistically significant differences at $p < 0.05$

**Data that exhibited statistically significant differences at $p < 0.01$

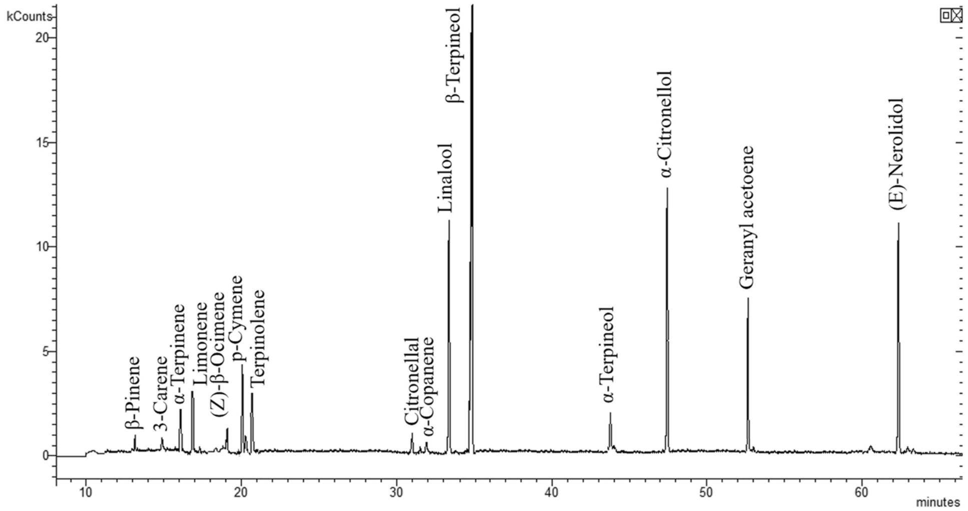

Fig. 1 Headspace Solid-Phase Microextraction–Gas Chromatography–Mass Spectrometry (HS–SPME–GC–MS) chromatogram in SIM mode ($m/z = 93.0 + 121.0 + 136.0$) of a Merlot wine sample

Table 4 OAV of the volatile compounds in Merlot wine samples

Compounds	2016		2017		ANOVA significance	
	Control	LR[a]	Control	LR	Tr[b]	Y[c]
Ethyl 2-methylbutanoate	1.25	1.90	1.73	2.41	*	*
Ethyl 3-methylbutanoate	30.16	36.35	36.16	41.87	*	*
Isoamyl acetate	12.37	21.50	12.30	21.43	*	ns[d]
Ethyl hexanoate	63.91	127.74	93.96	159.09	*	*
Ethyl octanoate	6.62	12.80	8.17	17.17	*	*
Methyl decanoate	6.59	18.33	6.51	18.52	*	ns
Ethyl decanoate	8.96	21.28	10.53	24.94	*	*
Isoamyl octanoate	0.42	0.55	0.41	0.56	*	ns
Hexanol	0.45	0.82	0.82	1.18	*	*
Octanol	0.50	0.83	0.50	0.83	*	ns
β-Phenylethyl alcohol	0.59	0.82	0.58	0.82	*	ns
Linalool	0.88	1.00	1.02	1.17	**	**

Odour activity values of compounds with values ≥ 0.50

[a] Leaf removal

[b] Treatment

[c] Year

[d] Non-significant

*Data that exhibited statistically significant differences at $p < 0.05$

**Data that exhibited statistically significant differences at $p < 0.01$

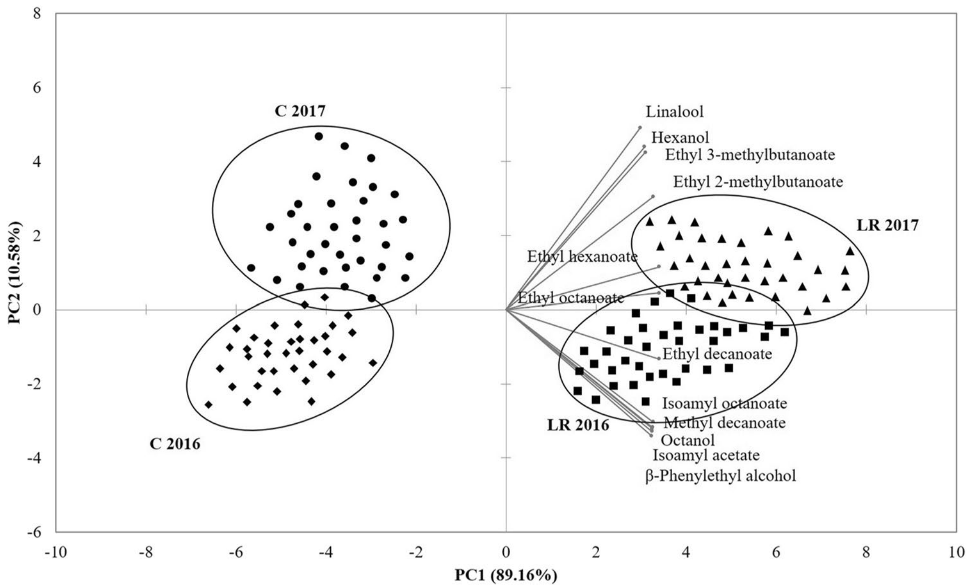

Fig. 2 Principal component analysis loading and score plot of volatile compounds with OAV values ≥ 0.5 of Merlot wine samples

10.58% for PC2). According PCA, wine samples from the two treatments were well separated along principal component one (PC1), whereas samples from the two vintages were quite close each other along principal component two (PC2) (Fig. 2). The variables that had the greatest effect on the separation of the analysed samples were ethyl ester from C_6 to C_{10}, isoamyl acetate, isoamyl octanoate, methyl decanoate and alcohols, such as hexanol and octanol, linalool and β-phenyl ethanol.

Discussion

The results underlined that both treatment and vintage affected yield and chemical composition of grapes and wines.

Regarding yield, previous studies have demonstrated that LR has changeable effects depending on timing and severity. Indeed, when defoliation is applied before flowering, a reduction in berry weight and, consequently, in fruit yield is observed [26], whereas late defoliation has an impact mainly on primary and secondary metabolite synthesis [27]. Also in this experiment, LR did not affect the number of bunches/plant whereas positively influenced the bunch and berry weight in both vintages. This can be related to plant evapotranspiration that is favoured by the higher canopy surface of the non-defoliated vines, accounting for the lower bunch and berry weight of the control. Regarding the vintage effects, the lower yield of 2017 was a consequence of a lower number of bunches per plant. The reduced number of bunches can be ascribed to the low rainfall of May and June months in 2017 vintage (Fig. 2S); in fact, it has been demonstrated that the water deficit occurring early in the season reduces bud fertility through falls in the number and size of inflorescences [28]. On the contrary, the absence of precipitation in August 2017 (after veraison) did not affect bunch and berry weight, as already observed by other authors [29–31].

The content of grape sugar (°Babo) was higher ($p < 0.05$) in samples from defoliated vines, whereas not statistically differences were observed between vintages. In our study, it is presumable that the small differences in the rainfall occurring from veraison to harvest in the two vintages, did not greatly modify the water soil conditions accounting for no vintage effect on °Babo values [32]. Regarding the treatment effects, the increasing in light availability at the cluster-zone induced by defoliation enhances grape quality, leading to grapes with higher sugar content and lower TA, and higher content of polyphenols and anthocyanins. The warmer and drier climate conditions of the 2017 vintage respect to 2016 had similar effects to LR on the total acidity, and on the total content of anthocyanins and polyphenols. Indeed, the water deficit occurring early in the season reduces vegetative growth, modifies the canopy microclimate and increases the amount of intercepted light in the fruit-zone [33].

Despite the lower total acidity content of grapes from 2017, especially from defoliated vines, no statistically significant ($p < 0.5$) differences were observed for the pH value, either between treatment or vintages. This behavior can suggest a different berry cation (Ca^{2+} and K^{+}) content [33].

Data on chemical and physicochemical parameters of Merlot wines highlighted the better quality of wine samples (lower total acidity content, higher anthocyanin and polyphenol contents) from defoliated treatment, especially in the 2017 vintage, as a consequence of the higher quality of the grapes. Moreover, the higher levels of anthocyanins and polyphenols accounted also for the higher values of color intensity and the lower value of color hue. The researchers reported in literature on the LR treatment applied to Merlot cv., mainly refer to vines which were cultivated under cool climate or hot climate; in either case the Authors demonstrated an increase in wine color intensity, and in anthocyanin and phenol concentrations when LR was applied [14, 16].

In addition, the wines from LR treatment showed a higher alcohol content due to the higher sugar content in grapes; similar results were reported also for various cultivars of *Vitis vinifera* [3, 4].

Regarding volatile profile, our wine samples, both control and defoliated, showed a composition more similar to that reported by Arcari et al. [24] who analysed samples from Brazil in a geographic area characterized by a temperate climate. In fact, for most of our volatiles the amount was included in the range reported by Arcari et al. [24], excluding for the varietal aromas and esters whose amounts were higher in our samples (esters for ex. showed an amount even up to five times higher in the defoliated samples).

The volatile profile of our Merlot wines showed distinctive peculiarities probably due to terror factors such as geographical area and climate as demonstrated by different Authors [22–25]. A higher amount of esters and varietal aroma compounds characterized the Merlot wines coming from the Mediterranean areas if compared with wines coming from different climate areas [22–25]. The increased amount of esters in the LR wines agrees with our previously research on the effect of this treatment on Nero d'Avola wine [3]; moreover, higher contents of esters were reported in the volatile profile of wines from vines grown under water deficit conditions [4, 29, 34], in agreement with the present findings. Esters are responsible of fruity notes, and it has been demonstrated that these compounds, such as ethyl hexanoate and ethyl 3-methylbutanoate, mainly contribute to the aroma of the Merlot wines [24].

Terpenes, classified as varietal compounds, have very low odour thresholds and even when present in low amount contribute to wine aroma being responsible for fruity and floral notes. For this reason, winemakers are very careful to increase the level of these compounds in grapes through various viticulture and winemaking techniques. Even if only linalool exceeded the odour threshold in our samples, the levels of varietal aromas increased following the LR treatment in agreement with Yue et al. [35], Feng et al. [36], and Alessandrini et al. [6], who demonstrated an increase of terpenes in Sauvignon Blank, Pinot noir, and Semillon wines, respectively. Similarly, the higher levels of varietal aromas in the 2017 vintage (warmer and dryer than 2016) agree with previous findings on wines from different cultivars, including Merlot [4, 29, 34], under water stress conditions.

Alcohols, such as hexanol and β-phenylethanol, and acids, such as butanoic and octanoic acids, showed a higher amount in the defoliated treatment with no significant differences between vintages.

The biosynthesis of volatile compounds in grapes is a very complex process; many factors, such as light intensity, temperature, water availability, and leaf removal, influence vine physiology and thus the content of volatiles in ripened grape [34].

Since water stress reduces vine vigor, it increases berry sun exposure and berry temperature, as it happens when leaf removal is applied after setting. Higher berry temperature and light exposure lead to an increase of grape content of monoterpenes, carotenoids, norisoprenoids, and aroma glycosides [4, 29, 37]; these glycoside-bound aroma compounds can be released during fermentation or aging and contribute, along with terpenes and norisoprenoids, to varietal aroma. Moreover, it is known that agronomic practices and climate conditions can have an impact also on the substances involved in the fermentation process [38]. This accounts for the resulting differences in the amount of most of volatiles, both fermentation and varietal ones.

Volatiles compounds are closely related to the sensory characteristics and contribute to the wine quality resulting determinants for consumer acceptability. The main contribution to the flavour is due to volatiles with concentrations higher than their OT, and in our wine samples esters, alcohols, and terpenes which characterize the olfactive notes of Merlot wines, were among these. The statistical elaboration of the data (PCA), allowed to distinguish the wine samples according to the treatment due to the higher amount of linear even number carbon atom ethyl esters (ethyl hexanoate, decanoate and octanoate) and alcohols (hexanol and octanol), the branched esters ethyl 3-methylbutanoate and ethyl 2-methylbutanoate, linalool and β-phenylethyl alcohol of LR samples mainly in 2017 vintage. The volatiles, above indicated, are responsible of fruity and floral notes and, as demonstrated by Pineau et al. [39], ethyl hexanoate and ethyl octanoate are involved in red berry aromas whereas ethyl 2-methyl butanoate in black-berry aromas. Thus, the LR applied to Merlot vine increased the berry fruit aroma, especially in warmer and dryer climate conditions, which is a desirable sensory feature of this type of wine.

Conclusions

The data here reported show how the application of the agronomic tool of LR on Merlot vines cultivated in a Mediterranean warm area, such as Sicily, can enhance the plant productivity, the chemical composition of both grapes and wine and, mainly, the aroma potential of Merlot wines. The results confirm those of our previous research on Nero d'Avola cv, further demonstrating that this agronomic technique can be successfully applied to red berry vines in the Mediterranean area.

It is important to underline that the vintage characterized by a lower rainfall enhanced the leaf removal effects on grape and wine quality. This makes our results even more relevant, since in the last years a global reduction of the summer rainfall has been registering in the Mediterranean area, as a relevant sign of the current climate alterations.

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Declarations

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